MISMATCH REPAIR ASSAYS ON PROTEIN ACTIVITY FOR USE IN SINGLE MOLECULE STUDIES

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Background

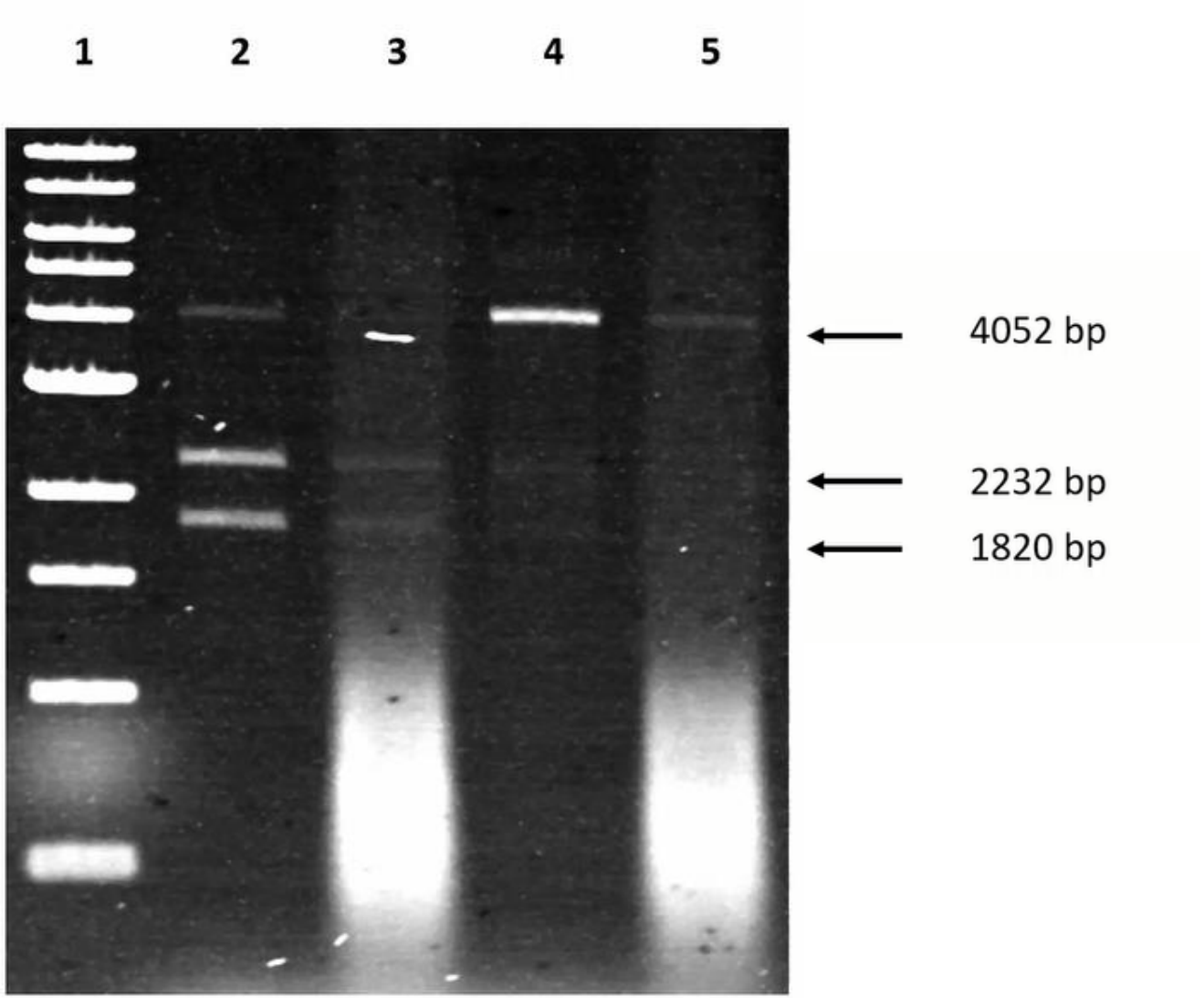
- Defects in the human mismatch repair (MMR) genes are the cause of Lynch syndrome as well as some other abdominal cancers
- The MMR system recognizes and repairs polymerase errors
- Lack of MMR results in increase mutations and tumorigenesis
- Single molecule methods are useful for determining MMR mechanisms
- Labeling for single molecule experiments may decrease activity of MMR proteins
- The goal of this project is to develop and assay that will evaluate the MMR activity of various mismatch substrates

Methods and Materials

- Obtained dsDNA
  - Maxiprep pT5 with the restriction site for XbaI
  - Column purified
- Obtained ssDNA
  - Has a thymine instead of a cytosine at the restriction site
  - Has f1ori site for ssDNA replication
  - Infected JM109 cells with MK13K07 bacteriophage
  - Pelleted culture and precipitate supernatant with PEG
  - Phenol:chloroform purified ssDNA
- Hybridized dsDNA and ssDNA
- Digested ssDNA with ExoI
- Nicked mismatched site on the T side with Nt. BbvCI
- Mixed cell extracts with mismatched DNA
- Quantified MMR activity with ScaI and XbaI restriction enzymes on a High Resolution 1% agarose gel

Results

Lane 1	1kb ladder
Lane 2	Homoduplex DNA without cell extracts
Lane 3	Homoduplex DNA with cell extracts
Lane 4	Heteroduplex DNA without cell extracts
Lane 5	Heteroduplex DNA with cell extracts



Results

- The results from the homoduplex and heteroduplex DNA are unclear
- Hybridization of the pT5-WT/pT5-GT produced bands appropriate for the restriction enzyme sites

Conclusions

- The optimization of the quantification step is still needed
- Whether the cell extract concentration or hybridization conditions require modification will be studied
- Must be tested on other types of mismatches
- Will be tested with other mismatch repair deficient cells and complementation with MMR proteins
- Applications in testing labelled MMR proteins